Case docket 20498
Patent Application

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Patent Application

Fukuda et al.

Group: 1625

Serial No: 09/702,944, filed 31 October 2000

Examiner: Patricia Morris

For: **N-Substituted Carbamoyloxyalkyl-Azolium Derivatives**

DECLARATION PURSUANT TO 37 C.F.R. §1.132

Nutley, New Jersey 07110

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

I, Isao Umeda, declare as follows:

1. I am Isao Umeda and I am an inventor of the invention described and claimed in the above-identified patent application. My postal address is Imperial Higashi Hakuraku Garden House (B) Room 513, Shirahata Minami-34, Kanagawa-ku, Yokohama-shi, Kanagawa-ken, 221-0073 Japan. I am employed by Nippon Roche Research Center, Japan, the assignee of the above-identified patent application.

2. I am familiar with the Examiner's Action dated 5 March 2002 in the above-identified patent application. In particular, I have reviewed the Examiner's position that United States patent no. 6,300,353 (*Hayase et al.*) discloses applicants' compounds in underivatized form and United States patent no. 6,265,584 (*Hudyama et al.*) teaches that analogous amine salts of triazoles retain the activity associated with the parent compound and

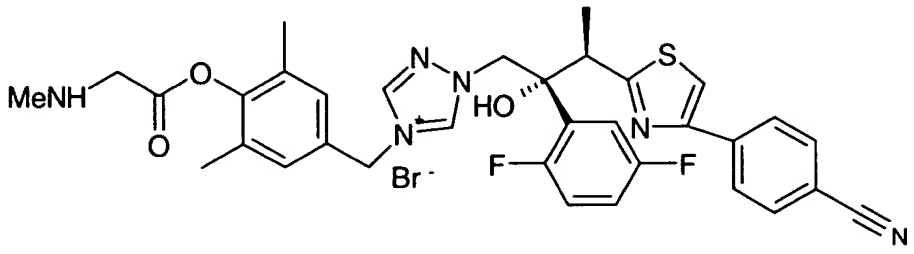
J. Med. Chem., 37, 4423-4429 (1994) (*Davidsen et al.*) teaches that pyridine salts are extremely soluble. (Examiner's Action dated 5 March 2002, p. 4).

3. In summary, we have unexpectedly discovered that the antigenicity of the N-substituted carbamoyloxyalkyl-azolium derivatives of the present invention are negative while the antigenicity of the corresponding prior art compounds, such as those of *Hayase et al.*, are positive. In the antigenicity study set out below, a series of tests was conducted in guinea pigs, in particular, active systemic anaphylaxis (ASA) tests and passive cutaneous anaphylaxis (PCA) tests were performed. Specifically, the antigenicity studies were performed on the closest prior art compound (pursuant to MPEP §716.02e) of *Hayase et al.* (compound 1, from example 1 on page 16 of *Hayase et al.*) and applicants' compound (compound 2, example c on page 66 of applicants' specification). No positive ASA reaction or PCA reaction was observed in animals immunized with 30 mg/animal of compound 2 plus Freund' s adjuvant (FA) and challenged with 1 mg/animal of compound 2 alone or with 1 mg/animal of compound 2-ovalbumin (OVA) mixture. No positive ASA reaction or PCA reaction was observed in animals immunized with 3mg/animal of compound 2 guinea pig serum albumin (GPSA) mixture plus FA and challenged with 1mg/animal of compound 2 alone or 1 mg/animal of compound 2-OVA mixture. The results from this study show that the antigenicity of applicants' compound 2 was negative. Active systemic anaphylaxis (ASA) tests on the prior art compound of *Hayase et al.*, compound 1, on the other hand, demonstrated that the antigenicity of compound 1 was positive. The details of this study are set out below.

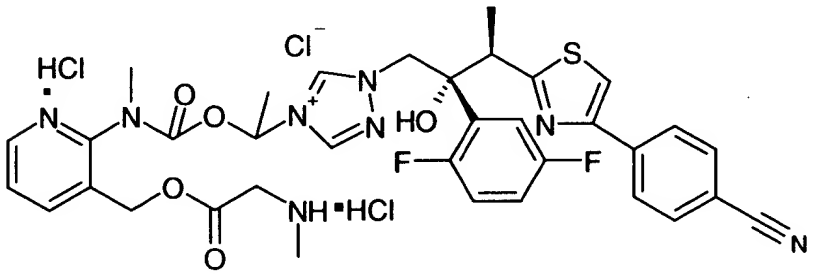
4. Under my direction, a series of active systemic anaphylaxis (ASA) tests and passive cutaneous anaphylaxis (PCA) tests were performed in guinea pigs. Specifically, the antigenicity studies were performed on the closest prior art compound of *Hayase et al.* (compound 1, from example 1 on page 16 of *Hayase et al.*), namely (1-{(2R,3R)-3-[4-(4-cyano-phenyl)-thiazol-2-yl]-2-(2,5-difluoro-phenyl)-2-hydroxy-butyl}-4-(3,5-dimethyl-4-methylaminoacetoxy-benzyl)-1H-[1,2,4]-triazol-4-ium bromide; and applicants' compound (compound 2, example c on page 66 of applicants' specification), namely 1-[[N-methyl-N-3-[(methylamino)acetoxymethyl]-pyridin-2-yl]-carbamoyloxy]-ethyl-1-[(2R,3R)-2-(2,5-

difluorophenyl)-2-hydroxy-3-[4-(4-cyanophenyl)thiazol-2-yl]butyl]-lH-[1,2,4]triazol-4-ium-chloride dihydrochloride.

The structure of compound 1 is set out below.



The structure of compound 2 is set out below.



The results of the active systemic anaphylaxis tests on applicants' compound 2 in guinea pigs are set out below.

Active Systemic Anaphylaxis (ASA) Tests of Compound 2 in Guinea Pigs

Group	Immunization	Challenge	ASA reaction (Positive/Total)
A	Compound 2-30 mg/animal + FA x 3	Compound 2-1 mg/animal	0/10

B	Compound 2-30 mg/animal + FA x 3	Compound 2-OVA mix.-1 mg/animal	0/10
C	Compound 2-GPSA mix. 3 mg/animal + FA x 3	Compound 2-1 mg/animal	0/10
D	Compound 2-GPSA mix. 3 mg/animal + FA x 3	Compound 2-OVA mix.-1 mg/animal	0/10
E	PCG 30 mg/animal + FA x 3	PCG-OVA conjugate 1 mg/animal	5/5

Immunization: The male guinea pigs were intradermally injected with each antigen with Freund' s adjuvant (FA) for 3-times with 2-week intervals.

Challenge: The immunized animals were intravenously injected with each antigen on the 8th day after the last immunization.

ASA reaction: The animals were observed for anaphylactic signs for 30 min after the challenging injection, and dead animals were necropsied. The positive reactions were defined by the death with a gross necropsy observation of the swelling of the lung. Because, anaphylaxis-like symptoms were observed in non-immunized guinea pigs intravenously injected with 1 mg/animal of Compound 2.

The results of the passive cutaneous anaphylaxis tests on applicants' compound 2 in guinea pigs are set out below.

Passive Cutaneous Anaphylaxis (PCA) Tests of Compound 2 in Guinea Pigs

Group	Immunization	Challenge	Sera dilution	PCA reaction (Positive/Total)
A	Compound 2-30 mg/animal + FA x 3	Compound 2-1 mg/animal	Undiluted	0/10
		Compound 2-OVA mix. (1 mg/animal)	Undiluted	0/10
B	Compound 2-30 mg/animal + FA x 3	Compound 2-1 mg/animal	Undiluted	0/10
		Compound 2-OVA mix. (1 mg/animal)	Undiluted	0/10
C	Compound 2-GPSA mix. 3 mg/animal (+ FA x 3)	Compound 2-1 mg/animal	Undiluted	0/10
		Compound 2-OVA mix. (1 mg/animal)	Undiluted	0/10
D	Compound 2-GPSA mix. 3 mg/animal (+ FA x 3)	Compound 2-1 mg/animal	Undiluted	0/10
		Compound 2-OVA mix. (1 mg/animal)	Undiluted	0/10
E	PCG 30 mg/animal + FA x 3	PCG-OVA conjugate (1 mg/animal)	1:100	5/5

Immunization: The male guinea pigs were intradermally injected with each antigen with Freund' s adjuvant (FA) for 3-times with 2-week intervals.

Blood sampling: The blood was sampled from the immunized animals on the 6th day after the last immunization, and the sera were isolated.

PCA test: The sera were intradermally injected in the nontreat guinea pigs, and challenged with each antigen on the second day from that. The diameters of the blue spots at the injection sites of the sera were measured. The positive PCA reaction was defined by an appearance of the blue spot with the diameter of 5 mm or more.

The results of the active systemic anaphlaxis tests in guinea pigs immunized with compound 1, the prior art compound of *Hayase et al.*, are set out below.

Active Systemic Anaphlaxis (ASA) Tests of Compound 1 in Guinea Pigs.

Test Group	Immunization Antigen	Dose	Challenge (Route: IV) Antigen	Dose	Grade of anaphlaxis signs 0	I	II	III	IV
A	Compound 1 + FA	30 mg x 3	Compound 1	1 mg	10	0	0	0	0
B	Compound 1 + FA	30 mg x 3	Compound 1 -OVA mix.	1 mg	5	3	1	0	0
C	Compound 1 -GPSA mix. + FA	3 mg x 3	Compound 1	1 mg	10	0	0	0	0
D	Compound 1 -GPSA mix. + FA	3 mg x 3	Compound 1 -OVA mix.	1 mg	3	6	1	0	0
E	PCG + FA	30 mg x 3	PCG OVA conjugate	1 mg	0	0	0	1	4
F	Compound 1 + FA	3mg x 3	Compound 1 -OVA mix.	1 mg	3	7	0	0	0
G	Compound 1 + FA	3 mg x 3	Compound 1 -OVA mix.	1 mg	5	4	0	0	0

Intensity (Grade):

0: Negative

I: Mild (restlessness, trembling, rubbing nose)

II: Moderate (sneezing & coughing, hyperpnoea)

III: Severe (respiratory depression, jumping & rushing, gasping & writhing, convulsion

IV Death

FA: Freund's adjuvant

GPSA: Guinea pig serum albumin

OVA: Ovallbumin

Compound 1-GPSA mixture (compound 1; 10 mg/ml, GPSA; 5 mg/ml): Incubation at 37°C for 16 hours

Compound 1-OVA mixture (compound 1; 10 mg/ml, OVA; 5 mg/ml): Incubation at 37°C for 16 hours

The results from the above Tables demonstrate that compound 1 is clearly antigenic and that applicants' compound 2 is unexpectedly lower in antigenicity and is therefore safer than the prior art compound of *Hayase et al.*, compound 1.

5. The materials and methods employed in these antigenicity studies are set out below.

1. Test Article

Compound 2 (Lot. MTS2111 for the main study and Lot. JO-10179 for the preliminary challenge dose-finding experiments) is a white powder (MW: 789.695). The chemical name of compound 2 is 4-[l-[(3-methylaminoacetoxymethyl-pyridin-2-yl)-methyl-carbamoyloxy]-ethyl]-1-[(2R,3R)-3-[4-(4-cyano-phenyl)-thiazol-2-yl]-2-(2,5-difluoro-phenyl)-2-hydroxy-butyl]-IH-[1,2,4]triazol-4-ium chloride dihydrochloride

2. Animals and Animal Housing

Male Hartley guinea pigs were supplied from Nippon SLC, Shizuoka, Japan. After one week quarantine, healthy animals (6-weeks old; body weight range: 342-441 g) were used for the study. The animals were housed individually in an air-conditioned room (room temperature: 22 ± 2 °C, relative humidity: 55 ± 10%). The animals were allowed free access to food (RC-4, Oriental Yeast Co. Ltd.) and tap water. Each animal was identified by picric acid marking.

3. Control and Reference Articles, and Other Materials

a) Penicillin G (PCG, Benzylpenicillin potassium) (Meiji Seika Co.Ltd.) was used as a positive control article.

b) Guinea pig serum albumin (GPSA) (Fraction V, Sigma) was used as a carrier protein in immunization.

c) Ovalbumin (OVA) (Grade VI, Sigma) was used as a carrier protein in challenge.

d) PCG-OVA conjugate (60 mg/ml) and OVA (10 mg/ml) were dissolved in 1M carbonate-bicarbonate buffer pH 10.4 and incubated at 37°C for 24 hours. The solution was dialyzed against 10 mM phosphate-buffered saline and stocked in a freezer.

e) Freund's adjuvant (FA). Complete Freund's adjuvant (CFA) and Incomplete Freund's adjuvant (IFA) (Difco Laboratories) were used as an adjuvant in immunization. CFA was used in 1st immunization. IFA was used in 2nd and 3rd immunization.

4. Immunization

Immunization was performed three times with 2-week intervals. Each antigen solution was injected intradermally into the dorsal region (0.2 ml/site x 3 sites) with Freund' s adjuvant. Immunizing antigens and doses are set out below.

Immunization

Group	No.	Immunizing Antigen	Dose	Adjuvant	Route
A	10	Compound 2	30 mg/animal x 3	FA	i.d.
B	10	Compound 2	30 mg/animal x 3	FA	i.d.
C	10	Compound 2-GPSA mixture	3 mg/animal x 3	FA	i.d.
D	10	Compound 2-GPSA mixture	3 mg/animal x 3	FA	i.d.
E	5	PCG (Penicillin G)	30 mg/animal x 3	FA	i.d.

The antigen solutions for immunization are set out below:

(a) Compound 2+ Freund' s adjuvant. Compound 2 was dissolved in physiological saline at the concentration of 100 mg/ml. The Compound 2 solution was mixed with an equal volume of Freund' s adjuvant and emulsified.

(b) Compound 2-GPSA mixture + Freund' s adjuvant. Compound 2 was dissolved in physiological saline at the concentration of 10 mg/ml. GPSA powder was added to the concentration of 5 mg/ml (Compound 2; 10 mg/ml, GPSA: 5 mg/ml) and incubated at 37°C for 16 hours. After the incubation, the solution mixture emulsified with an equal volume of FA (Compound 2; 5 mg/ml, GPSA: 2.5 mg/ml).

(c) PCG + Freund' s adjuvant. PCG was dissolved in physiological saline at the concentration of 100 mg/ml. The PCG solution was mixed with an equal volume of FA and emulsified (50 mg/ml).

5. Blood sampling

On the 6th day (Day 34) after the final immunization, blood was collected from eye (retro-orbital sinus) and sera obtained were used for PCA test. Sera were stored at 4°C until use.

6. Active systemic anaphylaxis (ASA) test

The ASA test was carried out on the 8th day (Day 36) after the final immunization in all animals. Challenging doses and routes are set out below.

ASA test

Group	Challenging Antigen	Dose	Route
A	Compound 2	1 mg/animal	i.v.
B	Compound 2-OVA mixture	1 mg/animal	i.v.
C	Compound 2	1 mg/animal	i.v.
D	Compound 2-OVA mixture	1 mg/animal	i.v.
E	PCG-OVA conjugate	1 mg/animal	i.v.

A) Antigen solutions for challenge are set out below:

(a) Compound 2 was dissolved in physiological saline (pH 3.5) at the concentration of 2mg/ml.

(b) Compound 2-OVA mixture. Compound 2 was dissolved in physiological saline at the concentration of 10 mg/ml. OVA powder was added in the solution to the concentration of 10 mg/ml (Compound 2; 10 mg/ml, OVA: 10 mg/ml), and incubated at 37°C for 16 hours. After the incubation, the solution mixture was diluted with physiological saline to the concentration of 2 mg/ml Compound 2 (Compound 2; 2 mg/ml, OVA; 2 mg/ml).

(c) PCG-OVA conjugate was diluted with physiological saline to the concentration of 2mg/ml.

B) Challenging injection for ASA reaction

0.5 ml/animal of each challenging antigens were injected intravenously into immunized animals on Day 36.

C) Evaluation of ASA reaction. For 30 minutes from the challenging injection, each animal was carefully observed for anaphylacitic signs described as follows.

Grade	Anaphylactic signs
0	no anaphylactic sign
I	restlessness, trembling, rubbing nose
II	sneezing & coughing, hyperpnoea
III	respiratory depression, jumping & rushing, gasping & writhing, convulsion
IV	death

However, some anaphylaxis-like syndromes (trembling, gasping, etc.) were observed even in non-immunized animals by the intravenous injection of 1 mg/animal of Compound 2. In the higher dose (2 mg/animal), the non-immunized animals died showing the anaphylaxis-like syndromes, but the lung didn't show a swelling which is a typical indication of anaphylaxis after the death. Therefore, the evaluation of positive ASA reaction was defined by the death with the swelling of the lung (gross observation). The grades of the reaction were not evaluated in this experiment.

7. Passive Cutaneous Anaphylaxis (PCA) Test

(2-day homologous in guinea pigs)

PCA test was carried out using sera obtained at the 6th day (Day 34) after the final immunization. Challenging doses and routes are set out below.

3 Homologous PCA test

Group:	Challenging Antigen	Dose	Route	Serum dilution
A	Compound 2	1 mg/animal	i.v.	undiluted
	Compound 2-OVA mixture	1 mg/animal	i.v.	undiluted
B	Compound 2	1 mg/animal	i.v.	undiluted
	Compound 2-OVA mixture	1 mg/animal	i.v.	undiluted
C	Compound 2	1 mg/animal	i.v.	undiluted
	Compound 2-OVA mixture	1 mg/animal	i.v.	undiluted
D	Compound 2	1 mg/animal	i.v.	undiluted
	Compound 2-OVA mixture	1 mg/animal	i.v.	undiluted

E	PCG-OVA conjugate	1 mg/animal	i.v.	1/100

A) Intradermal injection of sera into recipient guinea pigs. 50 mcl of undiluted or diluted sera were intradermally injected into the dorsal skin of recipient animals. Normal guinea pig serum and physiological saline were injected in the same manner as negative controls.

B) Antigen solutions for challenge.

(a) Compound 2was dissolved in physiological saline (pH 3.5) at the concentration of 2 mg/ml and mixed with an equal volume of 3% Evans blue in physiological saline.

(b) Compound 2-OVA mixture. Compound 2 was dissolved in physiological saline at the concentration of 10 mg/ml. OVA was added in the solution to the concentration of 10 mg/ml (Compound 2; 10 mg/ml, OVA: 10 mg/ml), and incubated at 37°C for 16 hours. After the incubation, the solution mixture was diluted with physiological saline to the concentration of 2 mg/ml Compound 2, and mixed with an equal volume of 3% Evans blue in physiological saline (Compound 2: 1 mg/ml, OVA: 1 mg/ml).

(c) PCG-OVA conjugate was diluted with physiological saline to the concentration of 2 mg/ml, and mixed with an equal volume of 3% Evans blue in physiological saline.

C) Challenging injection for PCA reaction. Challenging injection was performed on 2-days after the intradermal injection of sera. Challenging antigens with Evans blue were injected intravenously into recipient animals. 1 ml/animal of each challenging antigen solution was injected. Two recipient guinea pigs per test serum were used.

D) Evaluation of PCA reaction. Positive PCA reaction was defined by appearance of 5 mm or more diameter of blue spot at the injection site of sera in one or two recipients out of two recipients.

6. Results. No positive ASA reactions were observed in animals immunized with 30 mg/animal of Compound 2 plus FA and challenged with 1 mg/animal of Compound 2 alone (group A) or with 1 mg/animal of Compound 2-OVA mixture (group B). No positive ASA reactions were observed in animals immunized with 3 mg/animal of Compound 2-GPSA mixture plus FA and challenged with 1 mg/animal of Compound 2 alone (group C) or with 1 mg/animal of Compound 2-OVA mixture (group D). In the case of PCG (positive control

article, group E), severe ASA reactions (death with the swelling of the lung in all cases) were observed.

No positive PCA reactions were observed in animals immunized with 30 mg/animal of Compound 2 plus FA and challenged with 1 mg/animal of Compound 2 alone or with 1 mg/animal of Compound 2-OVA mixture (group A and B). No positive PCA reactions were observed in animals immunized with 3 mg/animal of Compound 2-GPSA mixture plus FA and challenged with 1 mg/animal of Compound 2 alone or with 1 mg/animal of Compound 2-OVA mixture (group C and D). In the case of PCG (positive control article, group E), positive PCA reactions were observed in all animals.

7. Conclusion. The results of this study show that the antigenicity of the N-substituted carbamoyloxyalkyl-azolium derivatives of the present invention are negative while the antigenicity of the corresponding prior art compounds of *Hayase et al.* are positive. Accordingly, the studies set out in this declaration show unexpected results in accord with MPEP §716.02(a).

8. All statements made herein of my knowledge are true and all statements made on information and belief are believed to be true. These statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing on this application.

Applicant requests the Examiner to telephone the undersigned attorney should the Examiner have any questions or comments which might be most expeditiously handled by a telephone conference. No fee is deemed necessary in connection with the filing of this Declaration. If any fee is required, however, authorization is hereby given to charge the amount of such fee to Deposit Account No. 12-2525.

Respectfully submitted,
Applicant

Dated this 15th day of July, 2002
Signed at Kamakura

By Isao Umeda
Isao Umeda

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